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13. ABSTRACT (Maximum 200 Words)

Murine models of prostate cancer have been developed that rely on the somatic activation of β-catenin. The approach employs Cre-loxP mediated targeted genetic recombination of the $Catnb^{+/lox(ex3)}$ locus. Expression of Cre was targeted specifically to the prostate secretory epithelium using androgen responsive minimal probasin (PB) or prostate specific antigen (PSA) gene promoters. We were able to demonstrate that the target of transformation by β-catenin in the prostate is the secretory epithelia. We have provided evidence for the benign nature of transformation by β-catenin and the conversion of this benign phenotype to invasive cancer upon heterozygous loss of PTEN. Local inflammatory reactions were shown to be inherently associated with and contribute to the local tumor microenvironment, suggesting a crosstalk between tumor and host immune response that may be contributing to the success of the tumor. Future work will focus on the contribution of the PTEN mutation to tumor progression, the contribution of local inflammatory responses, and studies of downstream targets of β-catenin in the prostate physiology and cancer.

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Initiating events in prostate cancer: The role of somatic activation of β-catenin.

Introduction.

Histopathological studies of prostate cancers have led to the identification of prostate intra epithelial neoplasia (PIN), a specific type of lesion that represents the primary precursor of human prostate cancer (2). PIN is recognized as a continuum between low-grade and high-grade forms, with high-grade PIN most likely representing the immediate precursor of early invasive carcinoma. Characteristic architectural and cytological features are shared between PIN lesions and early invasive carcinomas including multifocal nature of the lesions, and common chromosomal abnormalities (reviewed in (3)). We have reported characteristic appearance of PINs upon stabilization of β-catenin in the prostate (4). This observation is in line with the deregulation of PI3kinase activity in prostate cancer (5), and recent observations that link PI3kinase activity with the accumulation of β-catenin and stimulation of the androgen receptor signaling (6, 7). In our earlier study, stabilization of β-catenin was achieved through Cre/loxP mediated excision of the third exon of β-catenin. This model was based on the MMTV-LTR driven expression of Cre in a wide range of secretory epithelia, and skin. Preneoplastic lesions were observed only in the prostate, suggesting a specific role for β-catenin in the initiation of prostate cancer. The research underway aims to clarify the role of β-catenin in prostate cancer, its relevance to human prostate cancer, and the cross talk of this signaling pathway with other events associated with human prostate cancer.

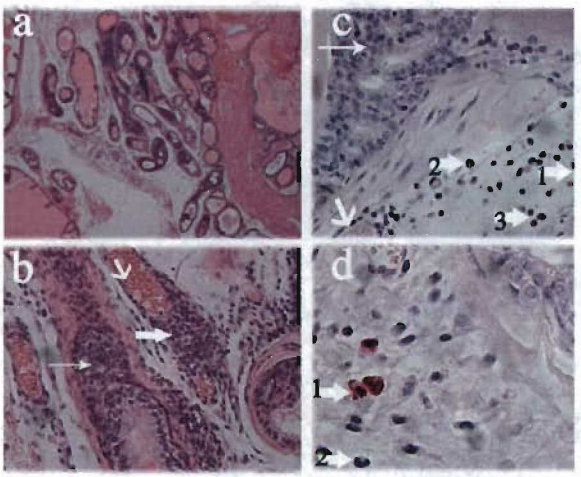

Figure 1. Prostatic lesions observed in PSA-Cre x Catnb$^{+/lox(ex3)}$ mice. a) x10 magnification. B) x20 magnification, showing neoangiogenesis (top arrow), proinflammatory cell infiltrates (thick arrow), and tumor mass (thin arrow). C) x60 magnification showing proinflammaory cells in tumor stroma, 1: mast cells, 2: neutrophils, 3: monocytes; thin arrow on top points to tumor mass, and below to a nearby blood vesse. Note within tumor areas, nuclear polymorphism, hyperchromia, and prominent nucleoli. In comparison, the neighboring healthy secretory cells form a regular monolayer, with underlying elongated basal cells. D) x100 magnification showing the morphology of prostate infiltrating proinflammatory cells, 1: mast cells, 2: neutrophils.

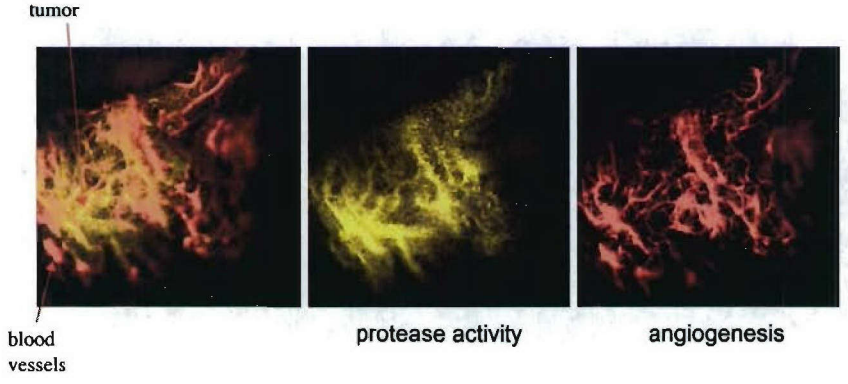

Figure 2. Imaging of cathepsin activity (yellow) and angiogenesis (red) in prostatic lesions caused by the stabilization of β-catenin. Left image is the superimposed version of the two images on the right. Mice were injected 24 before analysis with 2 nmoles of an activatable Cathepsin-B sensing probe, emiting at 694 nm, and just before imaging with a nonspecific fluorochrome emiting at 750 nm. The NIRF probe contained Cy5.5 monofunctional dye reporters adjacent to . . K-K . . . cleavage sites, on a macromolecular assembly. The assembly consisted of a synthetic graft copolymer containing partially pegylated (5 kilodaltons) poly-L-lysine (35 kilodaltons). The injection dose and time of imaging after injection had previously been optimized in APC$^{\Delta 468}$ mice bearing adenomas(1).

Body.

Task 1&2.

To establish the role of β-catenin in the initiation of prostatic intraepithelial neoplasia (PIN) like lesions, & determine targets of oncogenic action of β-catenin.

We have achieved prostate specific expression of Cre under the minimal probasin (PB) promoter (8), or the prostate specific antigen (PSA) promoter. The PSA-Cre mice were described before (9, 10). Both promoters are androgen dependent, while the PSA promoter is also transactivated by β-catenin.

These mice were crossed with Catnb$^{+/lox(ex3)}$ mice, and male progeny of 3 months of age were analyzed for prostate lesions. Double transgenic male progenies develop PIN, as revealed by histologic analysis. The PB-Cre mice generate less aggressive and more limited disease, in comparison with the PSA-Cre mice, which appear to have more extensive lesions, with profound inflammatory infiltrates (Fig. 1). The PB-Cre or PSA-Cre mice do not generate lesions in other tissues, and subsequently the mice are otherwise in good health.

Loss of the adenomatous polyposis coli (APC) gene is considered to be

the genetic cause of human colon cancer. In contrast to frequent APC defects, mutations affecting the β-catenin gene are rare in colon cancer. These facts suggest that although the stabilization of β-catenin efficiently promotes pre-neoplasia, by itself it may not be sufficient to cause malignancy. In a collaborative study with Dr. Fotini Gounari we have begun to gain insight into this issue, by comparing the impact of mutations that lead to the loss of APC with those that directly stabilize β-catenin. By introducing the corresponding Cre dependent conditional mutations in lymphocytes, we have shown that loss of APC leads to blockage of mitosis and that escape from this block requires aberrant chromosome segregation. As a result dividing cells that are deficient for APC are inherently genetically unstable. In contrast, direct stabilization of β-catenin leads to aberrant proliferation and differentiation of immature lymphocytes. These observations indicate that genetic instability associated with the loss of APC may be a critical factor in promoting the malignant transition of the lesions, and that the stabilization of β-catenin in the presence of intact APC function may therefore not be sufficient for tumor progression (manuscript submitted).

We are currently comparing prostate specific loss of APC with stabilization of β-catenin to gain further insights into the role of genomic instability in tumor progression in the prostate. So far, we have confirmed occurrence of PINs in mice that have lost APC function in a prostate specific manner. Further work is underway to compare proliferation and genetic stability of prostate epithelial cells in the two types of mice, and to relate these to the ability of the lesions to progress to malignancy.

The lesions caused by PSA-Cre appear to be massively infiltrated with inflammatory cells. We had earlier reported the use of protease activatable fluorescent probes for imaging of adenomatous polyps (1). The presence of proinflammatory leukocytes in the prostatic lesions encouraged us to apply similar imaging techniques to the prostate. To this aim 3-7 months old PSA-Cre x $Catnb^{+/lox(ex3)}$ mice were injected with Cathepsin-B sensitive probe with emission maximum of 694, 24 hours in advance. The next day the mice were anesthetized, injected with a fluorescent polymer that emits at 750 nm and reveals blood vessels, and prostates were imaged by intra vital fluorescent confocal microscopy using a x4 lens. The imaging analysis revealed *in situ* Cathepsin activity in diseased prostates, with abundant neo-angiogenesis supplying the lesions (Fig. 2).

To reveal the cellular source of the proteolytic activity in the lesions we prepared single cell suspensions of the entire diseased prostates by limited collagenase digestion, and separated the epithelial from the leukocyte cell populations using percoll gradient centrifugation. Cell surface staining and FACS analysis revealed CD11b+Gr1+ cells as the cellular source of the Cathepsin signal. These observations suggest that stabilization of β-catenin and initiation of prostate cancer coincide with local inflammatory reactions that contribute to the elevated proteolytic activity of the tumor micoenvironment.

Task 3.

To test the hypothesis that β-catenin induced lesions can progress to carcinomas. The cooperation of PTEN/Akt-1 pathway with β-Catenin.

Lesions produced by the stabilization of β-catenin in the prostate are preneoplastic and non-invasive, suggesting that additional genetic mutations may be required for tumor progression. PTEN (Phosphatase and tensin deleted on chromosome 10) mutations have been frequently associated with progression in prostate cancer, and are present in 30% of

primary (11) (12) and 63% of metastati prostate cancers (13). In mice, prostate specific loss of PTEN predisposes the animals to metastatic prostate cancer (14) (15) (16). Interestingly genetic deficiency in PTEN in humans is associated with at least two hereditary Hamartoma disorders, Cowden's syndrome (CS) and Bannayan-Riley-Ruvalcaba syndrome (BRRS). These are typically diseases of inflammatory nature with predisposition to cancer. It was therefore postulated that deficiency in PTEN may promote malignant progression of the β-catenin induced PINs, and that the progressive tumors would have an inflammatory nature. To test this, we introduced a heterozygous PTEN deficient allele in PBCre x Catnb$^{+/lox(ex3)}$ mice. At 3 months of age mice containing all three mutations harbored invasive prostate cancer with a strong inflammatory component (Fig. 3). Imaging of the mice with Cathepsin activated fluorescent probes revealed strong proteolytic activity within the prostate. Imaging of the vasculature feeding the lesions revealed abnormally expanded and thin walled vessels, characteristic of tumor vasculature. Rapid leakage of the imaging probe indicated leakiness of the vessels, which is also characteristic of blood vessels in advanced tumors (Fig. 4).

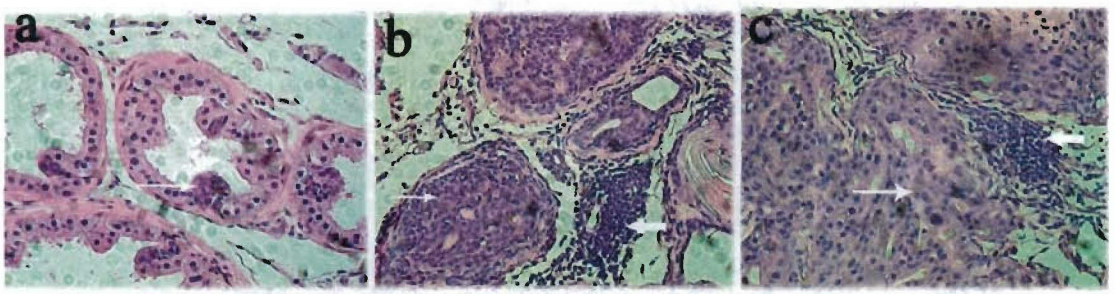

Figure 3. Progression from benign PIN in PBCre x Catnb$^{+/lox(ex3)}$ mice to invasive carcinoma in PBCre x Catnb$^{+/lox(ex3)}$ x PTEN mice. a) typical Pin lesions in the prostate of double transgenic mice at 3 months of age, b) and c) histologies of prostates from two independent PBCre x Catnb$^{+/lox(ex3)}$ x PTEN mice of 3 months of age. Note absence of inflammation in (a) but profuse inflammatory infiltrates in (b) and (c). Thin arrow point to tumor, thick arrows to inflammatory infiltrates.

These observations are consistent with deregulation of β-catenin in primary prostate cancer, and with the loss of PTEN contributing to tumor progression. Furthermore they implicate inflammation in the process of tumor progression. Recent works from several laboratories have suggested that inflammation may play a determining role in facilitating the growth and progression of tumors. In transgenic models of skin cancer inhibition of proteolytic activity associated with tumor infiltrating pro-inflammatory leukocytes, or depletion of CD4+ T cells leads to significant delay and suppression of tumorigenesis (17-19). These reports implicate host inflammatory responses in the acceleration of tumor progression. In mouse models of colon cancer CD4+CD25+ immune suppressor cells play a significant role in slowing down the growth of adenomas (20, 21). Our

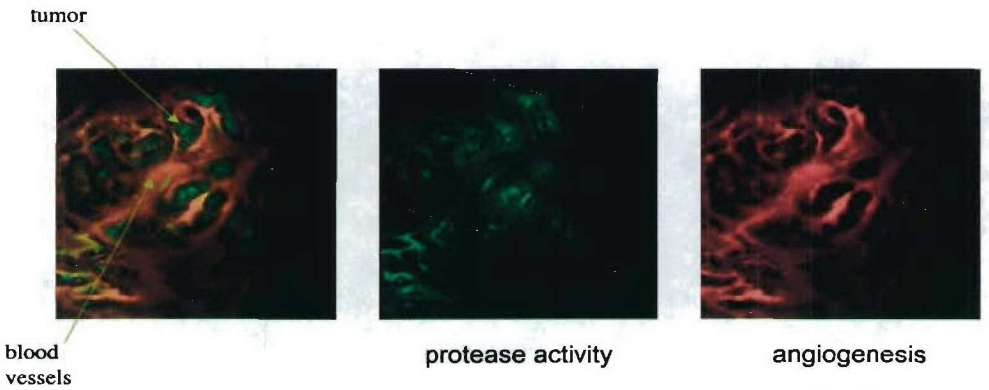

Figure 4. Imaging of prostatic lesions from a 3 months old PBCre x Catnb$^{+/lox(ex3)}$ x PTEN mouse. The images here are from the same mouse and correspond to the histology shown in Figure 4b. The mouse was injected with probes for imaging cathepsin activity and angiogenesis and mice were imaged, as described in Figure 3. Note extensive areas with Cathepsin activity and distorted image of supplying blood vessels, due to leakage of the probe from the vessels. The image on the left is an overlapped image of the two images on the right. Cathepsin activity is pseudo-colored as green, and blood vessels as red.

unpublished results suggest that this may be due to the suppression of innate inflammatory responses to the intestinal lesions. The major components of these inflammatory reactions are macrophages, neutrophils and mast cells, the same cells seen to infiltrate prostatic lesions in our studies. Altogether, the reports and our unpublished observations suggest that stabilization of β-catenin and the cooperative effect of PTEN lead to the progressive growth of invasive prostate cancer, and that the extent of inflammation in the lesions may be predictive of the advanced/malignant nature of the lesion. We are currently testing these hypotheses by crossing our mice to Cathepsin-B deficient mice, as well as by manipulating the host responses to the tumors.

Key research accomplishments (2004-2005).

- It was demonstrated that stabilization of β-catenin in the prostate secretory epithelia, is responsible for the initiation of PIN.
- It was demonstrated that these lesions in the mouse do not progress to malignancy at least with the first 1/3 life span of the mouse.
- Preliminary observations suggest that the inability of the above lesions to progress may be related to the genetic integrity of aberrant cells; in contrast loss of APC leads to genetic instability.
- Stabilization of β-catenin was linked to local inflammatory reactions.
- Malignant progression of the β-catenin induced lesions was achieved by introducing a heterozygous defect in PTEN.
- Tumor progression was linked with increased inflammation and local proteolytic activity.

Reportable outcomes.

The first year focused our attention on improving and characterizing the animal models, as well as relating our observations to human prostate cancer. In the second year, we established the benign nature of transformation by β-catenin and addressed the possibility that this may be related to the genetic integrity of the lesions. We provided evidence for the cooperation of β-catenin signaling with PTEN deficiency in promoting malignant progression of prostate cancer. Furthermore, we linked tumor progression with local inflammatory reactions.

Conclusions.

Using different transgenic mice we were able to demonstrate that the target of transformation by β-catenin in the prostate is the secretory epithelia. We have provided evidence for the benign nature of transformation by β-catenin and the conversion of this benign phenotype to invasive cancer upon heterozygous loss of PTEN. Local inflammatory reactions are inherently associated with and contribute to the local tumor microenvironment, suggesting a crosstalk between tumor and host immune response that may be contributing to the success of the tumor.

Future work will focus on the contribution of the PTEN mutation to tumor progression, the contribution of local inflammatory responses, and studies of downstream targets of β-catenin in the prostate physiology and cancer.

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2005- Member of Committee for Immunology, Harvard Medical School
2005- Active Member of New York Academy of Sciences

Adhoc Reviewer:
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Zenke, M., **Khazaie, K.**, Beug, H. 1990. V-myc transformed macrophages expressing the normal human EGF receptor are induced to proliferate by EGF via a nonautocrine mechanism. In *Molecular Biology of Hematopoiesis*, Edited by L. Sachs, N.G. Abraham, C.J. Wiedermann, A.S. Levine, G. Konwalinka, pp 453-467. Intercept, Andover, Hampshire, Great Britain.

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Research projects completed or ongoing in the last three years (PI: K. Khazaie)

Ongoing Research Support (PI: K. Khazaie)

Idea Award. May 2002 - May 2005
DAMD17-02-1-0361, Department of Defense Breast Cancer Research Program,
Title: Cancer Immunology in an inducible model of breast cancer.
Major goals: To develop an animal model of inducible mammary cancer, and to use this model to study antigen specific immune responses against the mammary gland and mammary tumors.

Idea Award. 2003-2006
DAMD17-03-1-0210, Department of Defense Prostate Cancer Research Program,
Title: Initiating events in prostate cancer: The role of somatic activation of β-catenin.
Major goals: To evaluate the stabilization of β-catenin as an initiating event in prostate cancer.

Claudia Adams Barr Program in Cancer Research. Boston, MA. 2004-2006
Dana Farber Cancer Institute
Title: Mast cells and orchestration of local inflammatory reactions in colon cancer.
Major goals: To investigate the cross-talk between tumor epithelium and infiltrating mast cells.

RO1-CA104547-01A1 2004-2009
Title: Imaging Proteolytic Activity in Colon Cancer
Major goals: Image proteolytic activity in animal models of colon cancer, and apply imaging to detection of tumor status and biological response.

Completed Research Support
Senior National Research Council Award Jan 2003 – Aug 2004
National Cancer Institute
(Salary Support only)

Inter-programmatic Research Award. 2002-2004
Dana Farber Cancer Institute/Harvard Cancer Center
Title: An animal model for investigating immunosurveillance and immunotherapy in prostate cancer.
Major goals: To develop an animal model of prostate cancer based on the prostate specific activation of the APC/β-catenin pathway, and to use this model to study antigen specific immune responses against the prostate.

National Colorectal Cancer Research Alliance. (Award) 2001
Entertainment Industry
Title: An Animal Model for Designing Targeted Immune Intervention in Colon Cancer.
Major goals: To Investigate antigen specific immune responses in the healthy and neoplastic mouse intestine.

Hershey Prostate Cancer/Survivors Walk. (Award) 2001
Beth Israel Hospital
Title: somatic activation of β-catenin reveals a critical event in the initiation of prostate cancer.
Major goals: To Investigate the role of β-catenin in prostate cancer.

Applied for

RO1-CA112348-01 re-submitted in Nov 2004
Title: Inflammation in Colon Cancer: A Cause or consequence?
Major goals: Define the role of innate immune response in the initiation of polyposis and in progression of invasive carcinomoma.